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Responses of Largemouth Bass from Different Latitudes to Elevated Water Temperatures

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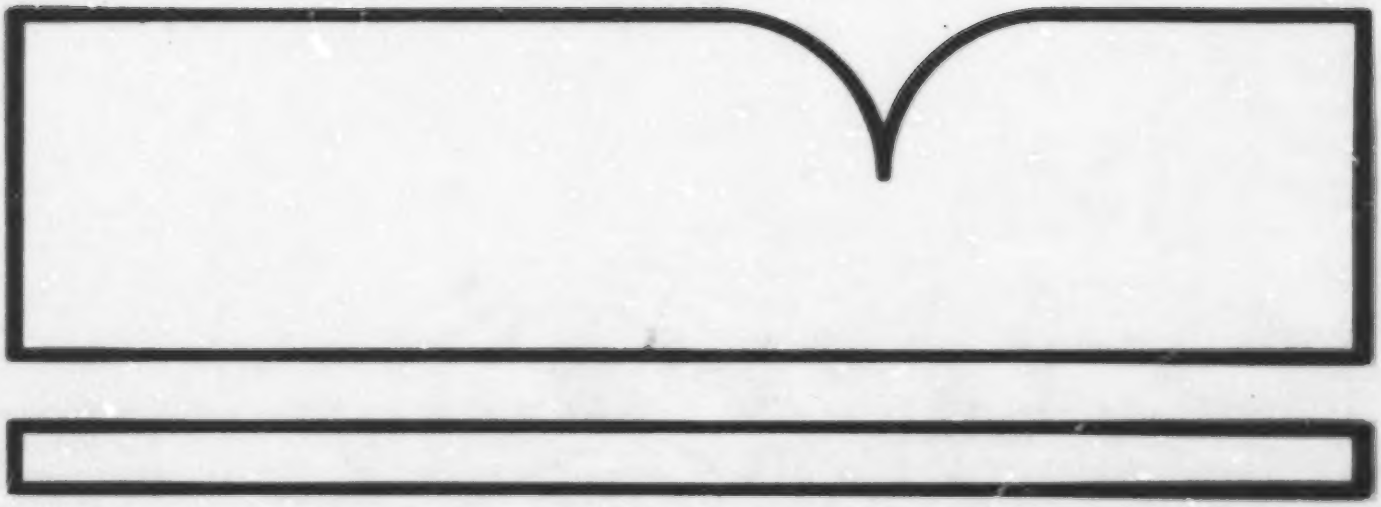

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16. ABSTRACT

The effects of elevated temperatures on largemouth bass (Micropterus s. salmoides), from Minnesota and Wisconsin (our northern stock) and from Tennessee (our southern stock), were compared at four first-year life stages. The purpose of these tests was to determine the degree of applicability of data gathered from a sample of fish from one part of its range of distribution to thermal standard setting for that same species at some other location within its normal range.

Eggs and embryos, when first exposed early in development to an array of elevated temperatures, lost 50% of their maximum hatching success (TL50) at 29.5 C for the northern stock and at 29.1 C for the southern stock. When embryos from the same pooled egg lots were exposed late in development, their TL50 temperatures were 32.3 C for the northern stock and 30.9 C for the southern stock. Median lethal 24-hour temperatures (TL50) for 0-3-day old larvae acclimated to 20, 24, 27, and 30 C averaged 32.1 C for the northern stock and 32.6 C for the southern stock. Median survival times for juveniles depended on acclimation temperature, exposure temperature, and the difference between these. Expected 24-hour TL50 temperatures, derived from median survival times, are 31.0 C for the northern stock and 30.6 C for the southern, based on 22 C acclimation. When reared at temperatures from 28 to 35 C for 6 weeks growth rates were not significantly different between temperatures or between stocks, but at 35 and 36 C for the northern and at 36 C for the southern stock mortality rates were significantly higher and net biomass gains were significantly lower than those at the most favorable lower test temperatures. The differences between stock tolerances exceeded 1 C in only one of the elevated temperature challenges performed (1.4 C for the TL50 of late-exposed embryos) and neither stock was consistently more or less thermally tolerant. We conclude that, in terms of responses to elevated thermal challenges, M. s. salmoides is a thermally consistent subspecies over the latitudinal range represented by our samples. We further conclude that thermal standards for these fish may be based on data obtained from samples taken from any usual population within this range, provided a 2 C safety factor is used where TL50 data are involved.

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Responses of Largemouth Bass from Different Latitudes to Elevated Water Temperatures

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Abstract

The effects of elevated temperatures on largemouth bass *Micropterus s. salmoides*, from Minnesota and Wisconsin (our northern stock) and from Tennessee (our southern stock), were compared at four first-year life stages. Our purpose was to determine if data gathered from one part of a species' range could be used to set thermal standards for that same species elsewhere within its normal range. Eggs and embryos, when first exposed early in development to an array of elevated temperatures, lost 50% of their maximum hatching success (TL50) at 29.5 C for the northern stock and at 29.1 C for the southern stock. When embryos from the same pooled egg lots were exposed late in development, their TL50 temperatures were 32.3 C and 30.9 C, respectively. Median lethal 24-hour temperatures (TL50) for 0–3-day-old hatched larvae acclimated to 20, 24, 27, and 30 C averaged 32.1 C for the northern stock and 32.6 C for the southern stock. Median survival times for juveniles depended on acclimation temperature, exposure temperature, and the difference between these. Expected 24-hour TL50 temperatures, derived from median survival times, are 31.0 C for the northern stock and 30.6 C for the southern, based on 22 C acclimation. When fish were reared at temperatures from 28 to 35 C for 6 weeks growth rates were not significantly different among temperatures or between stocks. At 35 and 36 C for the northern and at 36 C for the southern stock, mortality rates were significantly elevated and net biomass gains were significantly depressed, although neither variable differed between stocks ($P > 0.05$). The differences between stock tolerances exceeded 1 C in only one of the elevated temperature challenges performed (1.4 C for the TL50 of late-exposed embyros) and neither stock was consistently more or less thermally tolerant. We conclude that, in terms of responses to elevated thermal challenges, *M. s. salmoides* is a thermally consistent subspecies over the latitudinal range represented by our samples. We further conclude that thermal standards for these fish may be based on data obtained from samples taken from any usual population within this range, provided a 2 C safety factor is used where TL50 data are involved.

In *Water Quality Criteria 1972* (NAS and NAE 1973), proposed temperature criteria for fish are based on the thermal requirements of individual species and life stages. It is assumed that a species has similar thermal tolerances throughout its geographic range. The purpose of our study was to test this assumption more completely than has been done previously. The work pertains to the validity of broadly applying thermal-response data obtained from geographically restricted samples. Temperature criteria most often are applied when addition of heat to an aquatic environment is proposed, so we studied the responses of the test species to elevated temperatures only.

We used two geographic stocks of largemouth bass, *Micropterus salmoides salmoides* (Lacepède), a northern one from the Wisconsin-Minnesota area and a southern one from Tennessee, and compared their responses to high temperatures during their first year of life. This is the period during which year-class strength is believed to be established for this species (Murr 1956; Hubbs and Armstrong 1962; Kramer and Smith 1962).

The Florida subspecies, *Micropterus salmoides floridanus* (Lesueur), differs from the rest of the species in taxonomy (Bailey and Hubbs 1949) and, possibly, in response to temperature (Clugston 1964; Inman et al. 1977; Scott et al. 1978). We avoided stocks known to include genes from this subspecies.

Studies to examine within-species variations in fishes related to their origins previously have been undertaken by Hart (1952), Blaxter (1956), McCauley (1958), Clugston (1964), Ad-

dison and Spencer (1972), Hall et al. (1978), and others. Hart (1952) found that exposing largemouth bass to different living conditions is sufficient to bring about a difference in thermal responses, and taxonomic differences at least to the subspecies level also can be expected. However, other studies by Krumholz (1944), Hubbs (1964), Otto (1973), Gibbons and Sharitz (1974), and Hall et al. (1978) have produced data suggesting within-species changes, although Hall et al.'s conclusions since have been challenged by Mather and Silver (1980). The cautions of Fry (1957) regarding the influences of factors other than genotype on thermal responses must be considered when the differing results of these studies are evaluated. We have attempted to remove many of these complications by rearing our two stocks of test fish under conditions as nearly uniform as possible. Food, water, and lighting were the same for both stocks throughout the pretest periods.

Methods

The northern stock was studied in 1976. One pair of largemouth bass was taken from Anodanta Lake, northwestern Wisconsin; eggs were stripped and fertilized by the dry method (Davis 1956). A second pair, from the Genoa National Fish Hatchery, southwestern Wisconsin, spawned normally in our laboratory. For these in-laboratory spawnings, methods similar to those of Carlson (1973) were used. In 1977, five pairs of southern fish from Loudoun Reservoir, Tennessee, spawned in our laboratory. The study began with the fertilized eggs of these matings. Prespawning and spawning temperatures were 24 C.

Egg-Embryo Studies

Embryos were exposed to test temperatures in 4 × 4-cm glass jars with open tops and 0.23-mm-mesh stainless steel screen bottoms held off the bottom by glass legs to allow free circulation of water past the embryos. The water baths, 10 × 40 × 10 cm deep, received a continuous flow of untreated Lake Superior water at constant temperatures. Aeration gave additional water mixing, and a self-starting siphon at the drain end of each water bath provided an oscillating water depth to enhance flow-through conditions. Chemical composition of the water was similar to that reported by Biesinger and Christensen (1972): total hardness about 45 mg/liter; total alkalinity about 43 mg/liter; pH near 7.2. Dissolved oxygen was 93–107% saturation (rarely above 102%).

The embryo experiments followed multifactorial design that tested temperature effects due to (1) geographical origin of eggs, (2) development stage at first exposure, (3) pairs of spawning fish (two from Wisconsin and five from Tennessee), and (4) all interactions. Duplicate samples of 50–156 eggs from each female were given each temperature exposure. Eggs were exposed at either of two developmental stages: as soon after fertilization as they could be collected and subsampled (always less than 24 hours), and when the embryo's tail began to move freely over the yolk (about 17 hours before hatching [(Carr 1942]). The latter eggs were incubated at 20 C until they reached the test stage. Twelve nominal test temperatures covered the range 20–35 C (Table 1).

We measured percent hatch of viable larvae; dead and deformed (at 30× magnification) larvae were not considered viable (Volodin 1960; Kokurewicz 1969; Vladimirov 1975). Hatch data were normalized for each spawning pair by setting the highest hatch at any test temperature to 100% and expressing all others as a percentage of that hatch; this was done to eliminate differences in egg fertility among spawning pairs. Normalized values were converted to arcsin $\sqrt{\text{percentage}}$ and subjected to Tukey's multiple range tests (Steel and Torrie 1960). Median lethal temperatures (TL50: temperatures at which percent viable hatch is 50%) were calculated as described by Hokanson et al. (1973).

Larvae Studies

Larvae hatched from the embryo tests at 20, 24, 27, and 30 C were tested further; incubation temperatures were considered acclimation temperatures. Except for one group (noted in Table 3, footnote b), all embryos had been exposed to constant incubation temperatures (acclimation temperatures) since fertilization.

At hatching, normal larvae were transferred to 4 × 4 × 11-cm stainless steel screen walled vessels and exposed to an array of test temperatures below and above their TL50 temperature. At the end of the exposure period life or death (no heart beat) was determined at 30× magnification. Sample size was 10 larvae per

exposure chamber; most tests had at least two replicates. Fluctuating water depths, used for egg incubation, were not used for the larval exposures.

Twenty-four-hour TL50's were calculated by the trimmed Spearman-Karber method (Hamilton et al. 1977). No statistically significant effect of acclimation temperature on TL50 was found by linear regression ($P > 0.05$). Neither was there an effect due to age of the larvae at first exposure (<24 hours old or 3 days posthatch) on the resulting TL50 values (t-test; $P > 0.05$). Consequently, the effect of spawner origin (north or south) on TL50 values was assessed from northern TL50's for <24-hour-old larvae acclimated at 20, 24, 27, and 30 C, and southern TL50's for both <24-hours- and 3-day-old larvae acclimated to 20, 24, and 27 C.

Juvenile Studies

Juvenile largemouth bass, about 1 cm long, were reared in the laboratory from eggs or early juveniles. Northern and southern stocks were each divided into six groups and acclimated in 152 × 28-cm rectangular tanks for 4 or more weeks. These tanks were supplied with flowing Lake Superior water (2–3 liters per minute) at one of six constant temperatures, 10, 15, 20, 25, 30, or 34 C; each varied no more than 0.5 C from the water input to the drain end. Northern fish were a mixture of fish spawned in our laboratory from the Genoa, Wisconsin, brood stock and from brood stock from Howard Lake, Minnesota, spawned in Tennessee. The southern fish were from brood stock from Concord Cove, Fort Loudoun Reservoir, Tennessee; all were reared in our laboratory from hatching or from very young stage shortly after swim-up.

The fish were fed to satiation twice daily on frozen adult brine shrimp (*Artemia* sp.) thawed and mixed with a ground form of the meal-gelatin diet described by Peterson et al. (1967). The weights of the fish at testing time varied among groups largely because of growth at different acclimation temperatures and population density. The mean weight of northern fish at testing was 3.29 g, range 1.87–5.03 g; the southern fish weighed 3.91 g, range 1.90–9.55 g.

Median survival times were determined for juveniles during exposure to elevated temperatures in 20 × 60-cm × 15-cm-deep glass aquaria with temperature-controlled, continuously flowing water. Reported temperatures are grand means of hourly means during exposures, deduced from the recording charts. Dissolved oxygen measured just before tests was 93–105% saturation. Fish tested were sampled in a stratified random manner; sample size depended on availability of appropriately acclimated fish. The fish fasted overnight before their exposure to test temperatures. The fish were transferred directly into the exposure tanks, and feeding resumed after the first 2 or 3 hours of testing (Scott 1964). Fish were observed continuously for the first 3 hours or more, and at hourly intervals thereafter for 6 days. Death was defined as the cessation of opercular movements. Dead fish were placed in 10% formalin for later weight determinations.

Median survival times of the northern and southern stocks were compared by least-squares regression analysis (Zar 1974). The best fit to the data was achieved with equations that factored in the difference between acclimation and test temperatures (ΔT; Scott 1964) and had the form $\log_e MST = a - b(\Delta T) - C(T_t)$, where MST is median survival time in minutes; a is an intercept; b and C are constants; and T_t is the exposure temperature (C).

Other juveniles from the same broods were tested for growth and mortality rates at test temperatures of 30, 32, 34, 35, or 36 C. At the time of distribution to the test tanks, northern fish averaged 0.68 g and southern fish 1.05 g. The difference in initial size was not believed to affect the optimum and adverse-effects temperatures sought by this portion of the work. Growth rates of largemouth bass already are known. We sought in this work only to establish differences in critical response temperatures between the stocks.

The mean water flow rate to the test chambers was approximately 425 ml/minute at the beginning of the test. It increased as the fish grew, to about 750 ml/minute by the end of the exposures, to maintain the dissolved oxygen concentration above 80% saturation. At no time was the measured dissolved oxygen below 5.5 mg/liter. Dissolved oxygen averaged 90.5% saturation (range 80–103%) for northern fish and 88.4% (73–103%) for southern fish. Dissolved oxygen was measured weekly.

Two days before the test fish were transferred to test conditions, temperatures of their

TABLE 1.—*Percentage viable larvae hatched from eggs of northern and southern stocks of largemouth bass incubated at constant temperatures. Asterisks (*) indicate values significantly less than mean maxima (Tukey multiple-range test; $P < 0.05$).*

Stock		Nominal temperature (C) 20	24	26	27	28	29	30	31	32	33	Oppenheimer stage when first exposed (Rugh 1962)
Eggs exposed early in development												
Northern stock												
Temperature (C)												
Mean		20.0	24.0	26.2	27.1	28.0	29.0	29.7	31.0	31.7	32.7	
Range		17.8–21.0	21.7–24.4	25.5–26.7	25.1–27.4	27.0–28.4	28.0–29.3	29.0–30.2	29.6–31.6	29.7–32.0	29.4–33.4	
Percent hatch[a]												
Pair I[b]	A	83	82	93	93	91	78	17	0	0	0	2
	B	85	88	90	95	86	80	30	0	0	0	
Pair II[c]	A	41	42	44	41	44	42	37	[d]	0	0	12
	B	41	46	42	52	35	40	34	0	0	0	
Mean		63	64	67	70	64	60	29*	0	0*	0*	
Southern stock[e]												
Temperature (C)												
Mean		20.0	24.0	26.0	27.0	28.0	29.2	30.0	31.0	32.0	33.0	
Range		14.3–22.5	15.4–24.4	22.4–26.4	22.2–27.4	19.4–28.4	18.5–35.8	15.8–30.6	20.0–31.3	21.0–32.3	18.0–33.5	
Percent hatch[a]												
Pair I	A	92	98	96	94	98	86	42	0	0	0	15
	B	98	96	92	80	96	86	38	4	0	0	
Pair II	A	20	24	32	12	6	0	0	0	0	0	6–8
	B	22	20	26	10	6	4	0	0	0	0	
Pair III	A	96	82	78	80	64	14	0	0	0	0	8
	B	90	94	58	72	66	18	2	0	0	0	
Pair IV	A	46	52	50	42	30	10	2	0	0	0	7
	B	40	50	38	40	34	4	0	0	0	0	
Pair V	A	90	88	88	90	72	50	8	0	0	0	8
	B	88	84	80	80	68	46	26	0	0	0	
Mean		68	69	64	60	54*	32*	12*	0*	0*	0*	
Eggs exposed late in development												
Northern stock												
Temperature (C)												
Mean		20.0	23.9	26.3	27.1	28.0	29.0	29.6	31.0	31.8	32.8	
Range		17.8–20.5	21.7–24.1	25.9–26.7	25.1–27.4	27.0–28.4	28.0–29.3	29.0–30.2	29.6–31.3	31.5–32.1	32.2–33.3	
Percent hatch[a]												
Pair I[b]	A	86	91	94	94	87	96	96	91	97	0	24–25
	B	92	90	98	97	98	96	94	89	97	0	
Pair II[c]	A	66	54	53	58	52	55	65	73	60	0	24–25
	B	51	55	46	44	51	43	49	58	37	0	
Mean		74	72	73	73	72	73	76	78	73	0*	
Southern stock[e]												
Temperature (C)												
Mean		20.0	24.0	26.0	27.0	28.0	29.7	30.0	31.0	32.0	33.0	
Range		16.2–22.5	17.7–24.4	23.6–26.4	23.4–27.3	21.4–28.3	18.5–35.8	19.2–30.6	20.0–31.4	21.0–32.4	16.4–33.5	

TABLE 1.—*Continued.*

Stock		Nominal temperature (C) 20	24	26	27	28	29	30	31	32	33	Oppenheimer stage when first exposed (Rugh 1962)
Percent hatch[a]												
Pair I	A	92	93	94	96	94	94	50	16	0	0	24–25
	B	94	96	98	96	98	92	62	14	0	0	
Pair II	A	26	20	20	24	24	26	24	20	2	0	24–25
	B	20	28	22	20	22	20	16	10	2	0	
Pair III	A	68	72	100	74	86	54	56	40	10	0	24–25
	B	98	98	92	88	76	54	52	42	6	0	
Pair IV	A	46	52	58	46	60	42	36	34	12	0	24–25
	B	60	28	34	20	24	42	42	36	8	0	
Pair V	A	86	94	94	84	92	80	92	64	32	0	24–25
	B	84	98	96	92	92	91	94	74	32	0	
Mean		67	68	71	64	67	59	52*	35*	10*	0*	

[a] A and B are replicates.
[b] United States Fish and Wildlife Service, Fish Hatchery origin, Genoa, Wisconsin.
[c] Anodanta Lake, Bayfield County, Wisconsin.
[d] One replicate only; one was lost before total egg count. Esimated viable hatch was not greater than 1%.
[e] Concord Core, Lauden Reservoir, Tennessee.

rearing tanks were raised from 20–21 C to 25.5 C. After fish were distributed in a stratified random manner to exposure chambers (50 fish per test lot, two replicates per temperature for each latitudinal stock), temperatures were gradually adjusted to test temperatures. Thermal adjustment extended over a 6-day period; temperatures changed 1 C/day during the last 4 days. During this time dead fish were replaced with fish from the original stocks to keep the starting population size equal in all chambers. Spatial variations of temperature within test chambers were within 0.2 C when a centrally placed temperature probe recorded 36 C, and within 0.1 C at 28 C. Temperature control failed occasionally, but only briefly, and we do not consider these short-term temperature excursions a significant influence on the results. Test temperatures reported are the grand means of recorded hourly means. A columnaris (*Flexibacter columnaris*) outbreak occurred during the thermal-adjustment period, and all test lots were treated with 20–30 mg/liter neoterramycin and 200 mg/liter formalin. During the next 9 days, two more neoterramycin treatments and four flow-through formalin-malachite-green treatments were given (Leteux and Meyer 1972).

During the next 6 weeks, growth and mortality were studied. During this time the fish were fed to satiation five times per day, except three times daily on Saturdays and Sundays. The diet was a 2:1 weight ratio of mixed brine shrimp (thawed) and meal-gelatin plus 3% CMC binder. Samples of eight fish were taken from each chamber on the 7th, 14th, and 28th days, and all remaining fish on the last (42nd) day of the test. These samples were for later weight determinations. Instantaneous rates of daily growth, mortality, and net biomass gain were determined (Hokanson et al. 1973; Ricker 1975). Weights were wet weights of fish preserved in 10% formalin.

Temperature effects on these variables were tested by two-way analysis of variance and Tukey's multiple range test (Steel and Torrie 1960). Data for the 36 C test were excluded from analyses of variance because of excessive variance.

These data allowed calculation of the safe maximum weekly average temperature (MWAT) value as described in *Water Quality Criteria 1972* (NAS and NAE 1973):

$$MWAT = \frac{T(Op) + T(ZNG)}{2};$$

$T(Op)$ = the temperature (C) at which maximum net growth rate occurs;

TABLE 2.—*Multifactorial analysis of variance for normalized percent viable largemouth bass larvae hatched. Asterisks denote * P < 0.05 or ** P < 0.01.*

Source	Degrees of freedom	Sum of squares	Mean square	*F* ratio
Origin	1	2,952.204	2,952.204	1.10
Pairs within origin	5	13,394.869	2,678.974	16.97**
Replicates within pairs and origin	7	1,104.907	157.844	
Stage	1	24,898.105	24,898.105	212.37**
Origin × stage	1	779.645	779.645	6.64*
Temperature	9	132,649.667	14,737.741	125.70**
Origin × temperature	9	6,606.722	734.080	6.26**
Stage × temperature	8	18,166.022	2,270.753	19.37**
Replicates × row × temperature	8	5,041.303	630.163	5.37**
Residual	216	25,324.354	117.242	

[a] Origin = Wisconsin or Tennessee; Pair = parents of fertile eggs; Stage = stage of embryo development at first exposure to test conditions; Temperature = test temperature.

T(ZNG) = the upper temperature (C) at which zero net growth is predicted by extrapolation of the net-growth curve.

Responses to Temperature

Embryos

Hatching success of largemouth bass eggs was affected both by test temperature and by stage of embryo development when eggs were first exposed to test temperatures. Latitudinal origin of the parents did not significantly contribute to the thermal tolerance found (Table 2). Regardless of parental origin, eggs exposed soon after fertilization rarely produce viable larvae at temperatures above 30 C (Table 1). Those eggs first exposed shortly before hatching produced normal larvae at temperatures through 32 C.

The TL50 temperature, at which hatching success was reduced to 50% of maximum, was 29.5 C for northern and 29.1 C for southern eggs when exposed early in development; and 32.3 C and 30.9 C, respectively, for northern and southern eggs when exposed late in development (Table 1).

Larvae

The pooled mean TL50's were 32.1 C for northern larvae and 32.6 C for southern larvae (Table 3). These were not significantly different (*t*-test; 7 df; $P > 0.05$) so parental origin did not influence temperature tolerance of largemouth bass larvae.

TABLE 3.—*Median temperature tolerance limits (TL50) of larval largemouth bass from northern (Wisconsin) and southern (Tennessee) environments.*

Parental origin	Age posthatching when first exposed	Incubation temperature (C)	24-hour TL50 (C) (95% confidence limits)[a]
Northern	<24 hours	20	31.2 (30.6–31.8)
		24	32.4 (31.3–33.5)
		27[b]	33.0 (32.0–33.3)
		30	31.7 (31.1–32.2)
	Mean, <24 hours		32.1
	11–12 days (7 mm TL)	20	33.7 (33.0–34.3)
Southern[c]	<24 hours	24	32.8 (32.4–33.2)
		27	31.9 (31.5–32.3)
	3 days	20	32.0 (31.5–32.6)
		24	32.7 (32.5–33.0)
		27	33.6 (33.3–33.9)
	Mean, <24 hours–3 days		32.6
	Swim-up	20	32.5 (32.1–32.9)

[a] Trimmed Spearman-Karber method (Hamilton et al. 1977).

[b] Half the larvae in this group were incubated only 24 hours at 27 C before hatching.

[c] The 96-hour TL50 for southern larvae first exposed when <24 hours old was 32.9 C (95% confidence limits: 32.6–33.3 C).

Juveniles

Median survival times (Table 4) for juvenile largemouth bass from northern and southern stocks fit the following equations (values are ±2 SD):

TABLE 4.—*Median survival times of largemouth bass juveniles from northern and southern populations when exposed to acutely lethal temperatures in excess of their acclimation temperatures.*

	Northern fish			Southern fish		
Acclimation temperature	Exposure temperature (C)	Median survival time (minutes)	Mean fish weight (g) (sample size)	Exposure temperature (C)	Median survival time (minutes)	Mean fish weight (g) (sample size)
34 C	40.1	37.7	4.17 (10)	40.1	19.6	2.96 (20)
	38.9	78.6	4.51 (10)	38.9	100.7	2.84 (20)
	38.0	192.2	5.03 (10)	38.0	284.6	2.95 (20)
	36.9	619.3	4.93 (10)	36.9	1,121.0	2.98 (20)
	36.0	7,175.8	4.67 (10)			
30 C	39.0	13.8	1.87 (10)	39.0	22.0	3.89 (21)
	38.0	103.5	1.99 (10)	38.1	59.8	5.04 (21)
	37.0	239.8	2.17 (10)	36.9	149.0	5.35 (21)
	36.0	370.5	2.43 (10)	35.9	313.6	4.69 (21)
25 C				37.9	5.3	6.51 (13)
	37.1	4.4	3.89 (10)	37.0	9.9	7.68 (13)
	35.9	10.4	[a]	35.9	35.9	6.64 (13)
	35.0	44.0	2.84 (10)	35.1	94.9	6.99 (13)
	34.0	226.8	2.90 (10)	34.1	177.5	8.37 (12)
	33.0	1,428.7	2.57 (10)	34.1	225.2	9.55 (11)
20 C	34.0	8.5	4.46 (10)	34.0	6.1	3.27 (19)
	33.0	13.6	4.31 (10)	33.0	22.4	3.41 (19)
	32.0	69.3	3.45 (10)	32.5	23.6	3.50 (19)
				32.0	545.7	6.64 (18)
				31.9	1,019.4	4.22 (19)
15 C				32.0	4.6	2.38 (10)
	31.1	12.4	2.79 (15)	31.0	9.4	2.16 (20)
	30.1	111.8	3.14 (15)	30.0	45.5	2.28 (20)
	29.0	780.4	2.75 (20)	30.0	116.9	2.51 (13)
	29.0	1,147.6	3.20 (25)			
10 C				30.1	4.7	2.03 (17)
				29.0	9.7	1.90 (17)
				29.0	5.5	1.95 (17)
	28.0	13.9	3.00 (10)	28.1	14.1	2.29 (17)
	27.0	22.9	2.08 (9)	27.1	62.3	2.24 (18)
				27.0	78.9	1.90 (17)

[a] Lost.

$$\text{northern:}\quad \log_e MST = (32.9 \pm 6.5) - (0.644 \pm 0.131\Delta T) - (0.638 \pm 0.158T_t);\quad r^2 = 0.83;$$

$$\text{southern:}\quad \log_e MST = (28.0 \pm 5.8) - (0.561 \pm 0.110\Delta T) - (0.520 \pm 0.127T_t);\quad r^2 = 0.82.$$

These response lines are not significantly different ($F = 0.05$; df = 3,44), so the data were combined to yield:

$$\log_e MST = (29.25 \pm 3.71) - (0.585 \pm 0.071\Delta T) - (0.551 \pm 0.089T_t);\quad r^2 = 0.86.$$ [1]

[1] Two outliers 2 SD from the line were deleted.

The fit of this line was equally good over the entire range of the data.

We studied median survival times by Hart's (1952) methods. Our 95% confidence intervals for northern juveniles overlap Hart's data for largemouth bass from Put-in-Bay, Lake Erie, for acclimations of 20, 25, and 30 C. Median survival times for our southern stock for the same three acclimation temperatures are not significantly different from Hart's values for Wilaka, Florida (*M. s. floridanus*), samples.

By Tukey's multiple range tests, neither northern nor southern juveniles showed significant changes in growth rate over the tested temperature range, 28–36 C (Table 5). Mortality rates increased significantly for northern fish at 35 C and at 36 C, and for southern fish

TABLE 5.—*Mean instantaneous rates of growth, mortality, and net biomass gain of juvenile[a] largemouth bass from northern (Wisconsin-Minnesota) and southern (Tennessee) stocks. Study periods were 42 days after acclimation to test temperatures (except as noted). Asterisks denote statistical difference from results at the most favorable test temperature ($P < 0.05$).*

Variable	Nominal temperatures (C)					
	28	30	32	34	35	36
			Northern fish[a]			
Measured test temperature (C)[b] (Range)	28.0 (27.1–28.7)	30.0 (28.3–30.5)	32.0 (30.2–33.1)	33.9 (31.4–34.3)	35.0 (32.3–35.5)[c]	35.9 (33.2–36.5)[d]
Fish comprising final sample (replicates A, B)	(16, 18)	(16, 16)	(19, 19)	(16, 20)	(8, 3)	(4)
Growth (wet weight, %/day)	3.73	4.24	4.50	4.29	3.67	1.43[e]
Mortality (%/day)	0.07	0.00	0.00	0.00	4.68[c]*	17.65[d]*
Net biomass gain (wet weight, %/day)	3.66	4.24	4.50	4.29	−1.01[c]*	−16.07[d]*
			Southern fish[a]			
Measured test temperature (C)[b] (Range)	27.9 (26.2–28.4)	30.0 (28.8–31.7)	32.0 (30.1–33.8)	33.9 (31.9–34.6)	34.9 (32.2–35.6)	36.0 (34.9–36.5)
Fish comprising final sample (replicates A, B)	(16, 18)	(18, 17)	(17, 17)	(13, 15)	(5, 8)	(5, 8)
Growth (wet weight, %/day)	3.82	3.30	3.96	3.62	4.01	0.68
Mortality (%/day)	0.00	0.00	0.06	0.22	1.16[c]	5.96[d]*
Net biomass gain (wet weight, %/day)	3.82	3.30	3.90	3.40	2.85[c]	−5.28[d]*

[a] Initial sizes: northern, 0.68 g; southern, 1.05 g.
[b] Mean temperatures had 95% confidence limits of ≤0.1 C.
[c] 28-day experiment, mortality rate too high to provide adequate sample size at 42 days.
[d] 14-day experiment, mortality rate too high to provide adequate sample size at 42 days.
[e] Replicate A only, all fish died in replicate B before 14 days.

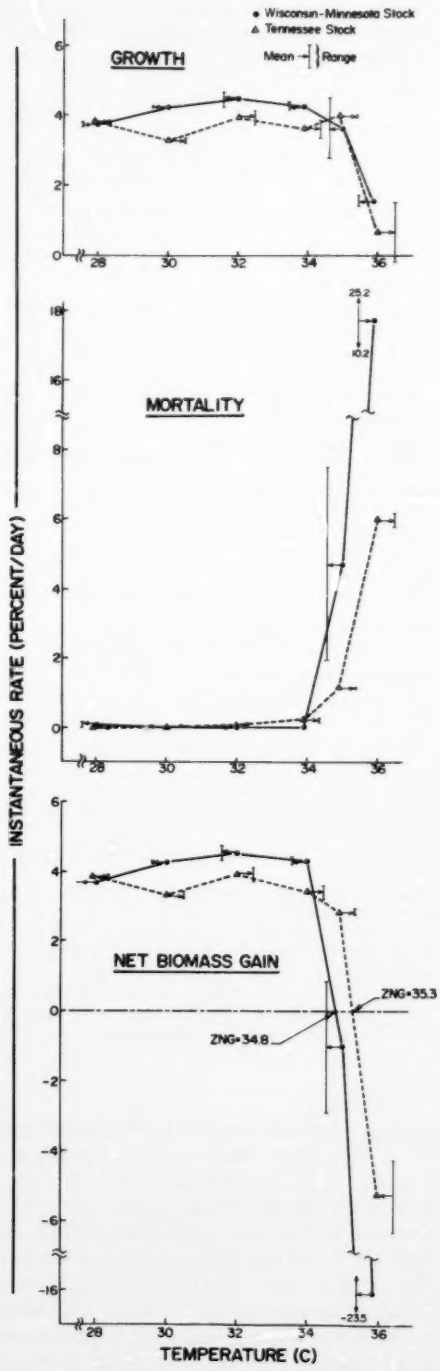
at 36 C; these caused significant losses of net biomass gain in each case. In spite of this, the rates of mortality and net biomass gain of northern and southern fish were not significantly different between stocks ($P < 0.05$). However, a significant interaction between fish origin and temperature did occur for net biomass gain, although to make these comparisons samples from less than the full 6 weeks of exposure had to be used. The shortened response times for the northern fish at 35 C and for both stocks at 36 C were necessary because high mortality rates at these temperatures provided too few survivors for dependable sample sizes at longer time intervals.

The difference in the mortality rates between the two stocks when they were exposed to 36 C was biologically significant. During the first week, few or no deaths occurred in either stock, but by the end of the second week, nearly all of the northern fish were dead, and all survivors would have been taken at the next scheduled sampling. The death rate was less severe among the southern fish, and declined after the second week in one replicate and by the third week in the other replicate.

By interpolation between net-biomass-gain data in Table 5, the temperatures of zero net growth T(ZNG), are 34.8 C for northern juveniles and 35.3 for southern fish (Fig. 1). The temperature for maximum rate of net biomass gain T(Op) was 32 C for both stocks. Thus, the safe maximum weekly average temperatures (MWAT) for northern fish (NAS and NAE 1973) are 33.4 C and 33.6 C for the southern fish, a 0.2 C difference.

Of the life stages of largemouth bass studied, the most sensitive to elevated temperatures were the embryos, particularly those exposed shortly after fertilization. Tolerance to high temperatures increased, however, with advancing development. It is important to note that these same stages were among those Kramer and Smith (1962) found to set year-class strength in largemouth bass. Precht (1973) also considered the sensitivity at these life stages critical to the limits of distribution of fishes.

Our findings and those of other workers studying largemouth bass are in relatively close agreement. Siler and Clugston (1975) reported juveniles at temperatures as high as 37 C, but most were found at 32 C, where growth was



highest. Gibbons et al. (1978), working at the same site as Siler and Clugston (1975), observed largemouth bass at 35 C with lower condition factors than those at cooler temperatures. Coutant and Cox (1975) reported zero net growth at 35.5 C. Maximum rates of growth in our studies were found at 32 C, a somewhat higher temperature than those observed by Strawn (1961) (27–30 C), and Coutant and Cox (1975) (26–28 C), but all within the temperature limits of our homogenous set of growth rates from 28 through 35 C, and the same temperature at which Siler and Clugston (1975) found greatest concentrations of fish.

When growth is incorporated into the rate of net biomass gain, the effect of mortality rate in removing biomass from the population reaches significance at 35 C among our northern fish, but does not become significant until 36 C among our southern fish. This effect was not found statistically different between the two stocks ($P < 0.05$, F-test), but if the effect were amassed over an entire growing season an important difference in production might be expected (Kitchell 1979). Even more potentially significant, though again not statistically so, is the onset of a severe death rate 1 week earlier among our northern fish at 36 C than among our southern fish. The importance of this is that lethal temperature for endemic species is most often episodic, and fish of the sensitivity of our southern stock would be more likely to survive a 36 C temperature excursion than would bass with the sensitivity of our northern stock.

Stock Differences

Certainly the ingredients for the development of geographic races are present in the largemouth bass. The isolation provided by the discontinuous nature of its lake habitats (Avise and Smith 1972) and distance (Avise and Smith 1977) in at least the major watershed where it is found provide for this potential. The wide range of the species also can be expected to contribute to differences within the species

←

FIGURE 1.—*Instantaneous rates of growth, mortality, and net biomass gain in northern and southern stocks of juvenile largemouth bass reared at different temperatures.*

(Davis 1977). In fact, differences have been reported within the species, and the subspecies *M. s. floridanus* is evidence of the differentiation that has already taken place in the isolation of the Florida peninsula (Bailey and Hubbs 1949; Hart 1952). Mayr (1970) suggests, however, that cohesion of a species may be retained through some homeostatic system that provides for species stability, and that evolution tends to be slowest in widespread species with large population size. The largemouth bass seems to possess these characteristics as well, and the elaborate courtship and spawning behavior of the species could also contribute to its stability. Behavioral isolation has been described as an isolating factor tending to maintain a species in a steady genetic state through elimination of unusual reproductive partners. Possibly most important are the vast state and federal stocking programs that have been in operation for some time. These provide outbreeding influences otherwise absent in isolated subpopulations and tend to keep the species similar throughout the range of introductions.

Of the comparisons of thermal-effect temperatures found in our test of northern and southern stocks of largemouth bass at several life stages, no statistically significant ($P \leq 0.05$) difference was found. Mathematical differences were maximum in the TL50 of the late embryo stage test, 1.4 C (Table 6), yet not statistically significant. All other comparisons found differences not greater than 0.5 C.

From the data contained in Table 6, an overall thermal-sensitivity comparison of the two stocks was made by a paired *t*-test and the Wilcoxon signed rank test (Steel and Torrie 1960). Neither test detected statistically significant differences between the two stocks ($P < 0.05$).

We thus conclude that, in terms of responses to elevated thermal challenges as conducted in our tests, *M. s. salmoides* is a thermally consistent subspecies over the latitude represented by our samples.

TABLE 6.—*Summary of temperature criteria for Wisconsin-Minnesota (northern) and Tennessee (southern) stocks of largemouth bass.*[a] *TL50 is median temperature tolerance.*

Response studied	Thermal criteria (C)		Safe standard (C) based on most tolerant sample[b]
	Northern stock	Southern stock	
TL50 egg hatch			
Early embryos	29.5	29.1	27.5
Late embryos	32.3	30.9	30.3
24-hour TL50 for ≤3-day-old larvae (acclimated 20–30 C)	32.1	32.6	30.6
Temperature producing 24-hour median survival time for 22 C acclimation	31.0	30.6	29.0
Temperature of maximum rate of net biomass gain[c]	32	32	
Temperature of reduced rate of net biomass gain	>34	>35	
Temperature of zero net growth (ZNG)	34.8	35.3	
Maximum weekly average temperature (MWAT)			
Based on zero net growth[d]	33.4	33.6	33.6
Based on ultimate incipient lethal temperature[e]	33.5	33.5	33.5

[a] Smirnov's nonparametric test of samples drawn from two possibly different populations and the paired *t*-test failed to show a significant difference between the temperature criteria for the northern and southern stocks (Steel and Torrie 1960).

[b] A 2 C safety factor is applied to all standards based on TL50 data.

[c] Maximum rate of net biomass gain is considered optimum for the MWAT calculations.

[d] Derived according to C. C. Coutant in NAS and NAE (1973), with our temperature for maximum biomass gain as optimum and our zero-net-growth temperature.

[e] Derived according to C. C. Coutant, with the UILT from Table III-12, NAS and NAE (1973), and our temperature for maximum net biomass gain as optimum.

Environmental Temperature Criteria

If the TL50 for early-exposed northern embryos were applied to environmental regulations without a safety factor, 29.6 C would be expected to kill more than half of any embryos as sensitive as our southern samples. The TL50 of late-exposed northern embryos (32.3 C) would endanger 90% of eggs as sensitive as our southern stock. Because a TL50 value implies loss of 50% of the stock, a safety factor seems in order. A 2 C safety factor has been recommended for another lethal variable, short-term exposure to extreme temperature (NAS nad NAE 1973), and its application to our embryo TL50 values seems warranted. Such a factor would adequately protect any of our largemouth bass embryos if a two-step criterion were recommended, one for early in the spawning

season and another for later in the season when all unhatched eggs are nearer hatching. However, because eggs of several stages of development as well as larvae can be expected to coexist at some time in the same environment, criteria probably should be for the most sensitive stage likely to exist at any time.

A 2 C safety factor, when applied to the TL50 values of larvae, also would be expected to protect larval largemouth bass of the sensitivity of either of our stocks. A trend toward slightly greater tolerance among larvae acclimated to 27 C than to 20 C suggests that standards could also allow for progressively higher temperature tolerance for larvae acclimated at successively higher temperatures from 20 to 27 C.

The importance of obtaining temperatures for the more long-term cumulative responses of rates of growth, chronic exposure mortality, and net biomass gain lies largely in their use in calculating the MWAT as proposed in *Water Quality Criteria 1972* (NAS and NAE 1973). Two methods are described, each requiring different data inputs.

The preferred method utilizes the formula

$$MWAT = \frac{T(Op) + T(ZNG)}{2}.$$

However, in the absence of *ZNG* temperature data (not commonly available for many species of fishes) the ultimate incipient lethal temperature (*UILT*) is suggested as a substitute, and the formula is modified to

$$MWAT = T(Op) + \frac{UILT - T(Op)}{3}.$$

In both approaches the derived MWAT values fall within the thermal limits where no significant reduction in rates of net biomass gain could be expected for either stock of our largemouth bass.

A final caveat in interpreting these findings is that all the results reported here deal with the response of the species to elevated temperatures. The limits of distribution of a species are, however, at least as likely to be set by cold tolerance as they are by heat tolerance (Hart 1952). Indeed, the disparity in tolerance between closely related populations may be expected to be greater on the cold end of the range (Kramer and Smith 1962) than on the warm end (Brett 1956; Shkorbatov 1966), but environmental controls on thermal perturbation have traditionally been restricted to heat addition.

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